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SPG11 mutations cause widespread white matter and basal ganglia abnormalities, but restricted cortical damage

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ABSTRACT

SPG11 mutations are the major cause of autosomal recessive Hereditary Spastic Paraplegia. The disease has a wide phenotypic variability indicating many regions of the nervous system besides the corticospinal tract are affected. Despite this, anatomical and phenotypic characterization is restricted. In the present study, we investigate the anatomical abnormalities related to *SPG11* mutations and how they relate to clinical and cognitive measures. Moreover, we aim to depict how the disease course influences the regions affected, unraveling different susceptibility of specific neuronal populations. We performed clinical and paraclinical studies encompassing neuropsychological, neuroimaging, and neurophysiological tools in a cohort of twenty-five patients and age matched controls. We assessed cortical thickness (FreeSurfer software), deep grey matter volumes (T1-MultiAtlas tool), white matter microstructural damage (DTI-MultiAtlas) and spinal cord morphometry (Spineseg software) on a 3 T MRI scan. Mean age and disease duration were 29 and 13.2 years respectively. Sixty-four percent of the patients were wheelchair bound while 84% were demented. We were able to unfold a diffuse pattern of white matter integrity loss as well as basal ganglia and spinal cord atrophy. Such findings contrasted with a restricted pattern of cortical thinning (motor, limbic and parietal cortices). Electromyography revealed motor neuronopathy affecting 96% of the probands. Correlations with disease duration pointed towards a progressive degeneration of multiple grey matter structures and spinal cord, but not of the white matter. *SPG11*-related hereditary spastic paraplegia is characterized by selective neuronal vulnerability, in which a precocious and widespread white matter involvement is later followed by a restricted but clearly progressive grey matter degeneration.

1. Introduction

Hereditary spastic paraplegia (HSP) is a diverse group of rare single-gene disorders that share key clinical aspects: progressive lower limb spasticity and weakness (Harding, 1983; de Souza et al., 2017). Mutations affecting the *SPG11* gene are the major cause of Autosomal Recessive HSP accounting for approximately 25% of the cases (Kara et al., 2016). The disease has a wide phenotypic variability indicating many regions of the nervous system besides the corticospinal tract are affected, but the anatomical basis of the disease is not well elucidated (Pensato et al., 2014; Siri et al., 2010).

Neuroimaging techniques have proven to be a powerful tool in

Abbreviations: ACE-R, Addenbrooke's Cognitive Examination Revised; ALS, amyotrophic lateral sclerosis; CA, cord area; CE, cord eccentricity; CMAP, compound muscle action potential; CST, corticospinal tract; DTI, diffusion tensor imaging; FA, fractional anisotropy; GM, grey matter; HSP, hereditary spastic paraplegia; LH, left hemisphere; MD, mean diffusivity; MOCA, Montreal cognitive assessment; NPI, neuropsychiatric inventory; PNP, sensory-motor polyneuropathy; PNS, peripheral nervous system; RH, right hemisphere; ROI, region of interest; SC, spinal cord; SNAP, sensory nerve action potential; SPRS, Spastic Paraplegia Rating Scale; STS, cortex adjacent to the superior temporal sulcus; WM, white matter; WES, whole exome sequencing

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many neurodegenerative disorders (Agosta et al., 2016; Hanganu et al., 2014). Such tools demonstrate *in vivo* structural abnormalities and provide a better understanding of the disease mechanisms and progression. Little is known about the extent of neurodegeneration in SPG11 patients and how it affects sequentially certain neuronal populations (França Jr et al., 2012; Pan et al., 2013).

Previous studies with limited number of patients have unraveled a diffuse pattern of White Matter (WM) microstructural integrity loss as well as basal ganglia atrophy. Most of these studies employed unimodal analyses focusing on WM tracts. Then, some unexplored aspects of the disease still deserve investigation, such as cortical and spinal cord involvement as well as potential correlations between clinical aspects and damage to specific brain regions (França Jr et al., 2012; Pan et al., 2013). In this scenario, we propose a comprehensive clinical, neuroimaging and neurophysiologic study regarding unexplored aspects of the disease in a cohort of 25 SPG11 patients. Our aim is first, to provide detailed genetic and clinical characterization concerning motor and cognitive aspects of the disease, correlating these findings with anatomical aspects. Moreover, we aim to delineate how the disease course influences the regions affected, unraveling different susceptibility of specific neuronal populations. To achieve these two objectives, we have applied robust neuroimaging tools to characterize unexplored anatomical aspects in the disease, such as cortical thickness, WM integrity, spinal cord area and morphometry.

2. Materials and methods

2.1. Subject's selection

Between 2013 and 2016, we enrolled 25 consecutive patients with confirmed *SPG11* mutations regularly followed at UNICAMP neurogenetics outpatient clinic. They came from 16 unrelated families. Molecular diagnosis was performed by Sanger and/or Whole Exome Sequencing (WES). Subjects underwent neurological, neurophysiological and neuroimaging examination as detailed below. None had associated neurological or systemic diseases.

Twenty-five age and sex matched healthy individuals were also included as a control group for neuroimaging analyses. None of them had past personal or family history of neurological disease.

This study was approved by our institution review board (CAAE 48122515.7.0000.5404), and all probands or their parents signed an informed consent before any study-related procedure.

2.2. Clinical and genetic assessment

Patients underwent a comprehensive neurological evaluation at the University of Campinas outpatient clinic: they were assessed for type and age of the first symptoms, associated neurological and clinical features. Cognitive disability, learning difficulties, as well as the presence of progressive cognitive deterioration were evaluated trough the Addenbrooke's Cognitive Examination Revised (ACE-R). The ACE-R is a comprehensive neuropsychological tool that has been validated for the Brazilian population. The score ranges from 0 to a maximum of 100 points (lower scores indicate greater impairment) and encompasses 5 cognitive domains: attention/orientation, memory, fluency, language and visuospatial abilities (Mioshi et al., 2006; Carvalho and Caramelli, 2007). Neuropsychiatric disturbances were explored through the neuropsychiatric inventory (NPI), a brief interview with the patient's main caregiver designed to detect behavioral issues such as anxiety, depression, and delusional thoughts (Cummings et al., 1994). Disease severity was quantified through detailed neurological assessment as well as the Spastic Paraplegia Rating Scale (SPRS) that ranges from 0 (no abnormalities) to 52 (Schüle et al., 2006). Such assessment was performed two times in 21/25 patients across a mean interval of 12 months (standard deviation: ± 2.06). The difference between the two SPRS measurements was used to assess the disease's rate of progression. All scales and examinations were performed by a single investigator (IF). For the genetic diagnosis, standard Sanger sequencing of the coding sequences and flanking intronic regions was performed in 13 families. WES was performed in five families (in two families one individual was submitted to Sanger and the other to WES). Methodological data on genetic diagnosis is available as supplemental material.

2.3. MRI acquisition and protocol

A 3 T Achieva PHILIPS scanner with a standard 8 channel head coil was used to scan all patients and controls. The exams were performed at the same day of the clinical evaluation. To exclude incidental findings, routine T1 and T2 weighted sequences were performed for all subjects. We obtained T1 weighted volumetric images covering the whole brain and the cervical spinal cord with the following acquisition parameters: sagittal orientation, voxel matrix $240 \times 240 \times 180$, voxel size $1 \times 1 \times 1\,mm^3$, TR/TE 7/3.201 ms, flip angle 8°. These T1-weighted images were used to measure spinal cord area/eccentricity, cortex thickness and deep GM volumes.

We also acquired a Gradient Echo Diffusion tensor imaging (DTI) sequence as follows: axial orientation, $2 \times 2 \times 2\,mm^3$ acquiring voxel size, interpolated to $1 \times 1 \times 2\,mm^3$; reconstructed matrix 256×256; 70 slices; TE/TR 61/8500 ms; flip angle 90; 32 gradient directions; no averages; max b-factor = $1000\,s/mm^2$; six-minute scan. The DTI images were used for analysis of WM integrity.

2.4. Image processing

2.4.1. Cerebral grey matter

2.4.1.1. Cortical thickness (n = 20 patients and controls). Cortical thickness was computed using the FreeSurfer software v.5.3. This tool is more sensitive to assess cortical damage than measures of area or volume (Hutton et al., 2009). Measurements were performed according to the protocol suggested by Fischl and Dale (2000) and have been previously detailed by our group (De Rezende et al., 2015). The software creates triangle meshes that form two surfaces, the interface between Grey Matter (GM) and cerebrospinal fluid (CSF) and the interface between GM and White Matter (WM). The shortest distance between the interfaces constitutes the measured cortical thickness. For comparisons, we followed the cortical regions as defined by Desikan et al. (2006). Five patients had their images excluded due to major segmentation errors. Those concerned patients with grossly enlarged ventricles due to ex vacuo atrophy and the main errors regarded labelling of lateral ventricles as WM or GM. Further FreeSurfer analyses were therefore performed with 20 patients and 20 controls.

2.4.1.2. Basal ganglia volumes (n = 21 patients and controls). T1 weighted images were processed with the T1 MultiAtlas approach using "MRICloud" (MRICloud.org), a public web-based service for multi-contrast imaging segmentation and volumetric quantification. T1 MultiAtlas provides an accurate and reliable segmentation of deep structures. Raw images were re-oriented (sagittal to axial), corrected for inhomogeneity and the whole brain was segmented after skull-stripping. Linear and non-linear algorithms (Miller et al., 2013) for brain co-registrations and a multi-atlas labeling fusion was employed to identify brain regions followed by a last step of labelling adjusting with PICSL (Tang et al., 2014). Nineteen atlases (JHU adult atlas version 9B) were used to generate 283 structural definitions (Wu et al., 2016). From these labels we were primarily interested in determining deep GM volumes. All analyses were performed in native space. The computations were processed on the Gordon cluster of XSEDE (Towns et al., 2014). Images from 4 patients were excluded due to minor segmentation errors. These occurred in the same patients in which FreeSurfer performed an inaccurate labelling with the exception of one patient whose image was successfully processed by T1-MultiAtlas but not by FreeSurfer. The paired controls were excluded with the objective